

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. An expression vector comprising:
 - (a) a first RNA polymerase III promoter operably associated with a first RNA polymerase III termination signal; and
 - (b) a second RNA polymerase III promoter operably associated with a second RNA polymerase III termination signal, wherein the first and second RNA polymerase III promoters are oriented to promote bidirectional transcription of an insert disposed between the first and the second RNA polymerase III termination signals.
2. The vector of Claim 1 further comprising a cleavage site for a restriction enzyme disposed within each of the first and second RNA polymerase III termination signals.
3. The vector of Claim 2 further comprising a recognition site for a restriction enzyme, wherein the cleavage site for the restriction enzyme is located outside the recognition site for the restriction enzyme.
4. The vector of Claim 2, wherein the cleavage site is cleaved by a restriction enzyme selected from the group consisting of Alw1, Bbs1, Bbv1, BceA1, BciV1, BfuA1, Bmr1, Bpm1, BpuE1, Bsa1, BseR1, Bsg1, BsmA1, BsmB1, BsmF1, BspM1, Ear1, Eci1, Fau1, Fok1, Hga1, Hph1, MboII, Mly1, Mnl1, Ple1, Sap1, and SfaN1.
5. The vector of Claim 4, wherein the restriction enzyme is BsmB1.
6. The vector of Claim 1 further comprising an insert disposed between the first and second RNA polymerase III termination signals.
7. The vector of Claim 6, wherein the size of the insert is between 19 and 29 nucleotides.
8. The vector of Claim 6, wherein the size of the insert is between 19 and 23 nucleotides.

9. The vector of Claim 1 further comprising a selectable marker operable in a eukaryotic cell.

10. The vector of Claim 1 further comprising an origin of replication operable in a eukaryotic cell.

11. The vector of Claim 1, wherein the vector is a plasmid vector.

12. The vector of Claim 1, wherein the vector is a viral vector.

13. The vector of Claim 1, wherein the vector is a linear vector.

14. A plurality of expression vectors, each comprising:

(a) a first RNA polymerase III promoter operably associated with a first RNA polymerase III termination signal;

(b) a second RNA polymerase III promoter operably associated with a second RNA polymerase III termination signal, wherein the first and second RNA polymerase III promoters are oriented to promote bidirectional transcription of an insert disposed between the first and the second RNA polymerase III termination signals; and

(c) an insert disposed between the first and second RNA polymerase III termination signals.

15. The plurality of vectors of Claim 14, wherein the size of the insert is between 19 and 29 nucleotides.

16. The plurality of vectors of Claim 14, wherein the size of the insert is between 19 and 23 nucleotides.

17. A method for inhibiting expression of a target gene, comprising introducing into a host cell an expression vector comprising:

(a) a first RNA polymerase III promoter operably associated with a first RNA polymerase III termination signal; and

(b) a second RNA polymerase III promoter operably associated with a second RNA polymerase III termination signal;

(c) a target gene-specific insert disposed between the first and the second RNA polymerase III termination signals, wherein the first and second RNA polymerase III promoters are oriented to promote bidirectional transcription of the target gene-specific insert to produce siRNA molecules that inhibit the expression of a target gene.

18. The method of Claim 17, wherein the size of the target gene-specific insert is between 19 and 29 nucleotides.

19. The method of Claim 17, wherein the size of the target gene-specific insert is between 19 and 23 nucleotides.

20. A method for determining the effect of an siRNA on a biological process, comprising the steps of:

(a) introducing into one or more host cells an expression vector comprising:

(i) a first RNA polymerase III promoter operably associated with a first RNA polymerase III termination signal;

(ii) a second RNA polymerase III promoter operably associated with a second RNA polymerase III termination signal; and

(iii) an insert disposed between the first and the second RNA polymerase III termination signals, wherein the first and second RNA polymerase III promoters are oriented to promote bidirectional transcription of the insert to produce siRNA molecules; and

(b) determining the effect of the siRNA molecules on a biological process of the one or more host cells.

21. The method of Claim 20, wherein the size of the gene-specific insert is between 19 and 29 nucleotides.

22. The method of Claim 20, wherein the size of the gene-specific insert is between 19 and 23 nucleotides.

23. The method of Claim 20, wherein the effect on the biological process is determined using a reporter gene.

24. The method of Claim 20, wherein the biological process mediates a biological signal transduction pathway.

25. The method of Claim 20, wherein the biological process mediates the expression of a cell surface molecule.

26. The method of Claim 20, wherein the biological process mediates stem cell differentiation.

27. The method of Claim 20, wherein the insert comprises a random sequence of nucleotides.

28. The method of Claim 20, wherein step (a) comprises introducing a plurality of expression vectors into one or more cells, wherein substantially all the vectors comprise a different insert.

29. The method of Claim 28 further comprising the step of identifying at least one insert from which siRNA molecules are transcribed that produce the effect on the biological process.

30. A method for identifying an siRNA that affects a biological process, comprising the steps of:

(a) introducing a plurality of expression vectors comprising a plurality of inserts into one or more cells, wherein each of the plurality of expression vectors comprises:

(i) a first RNA polymerase III promoter operably associated with a first RNA polymerase III termination signal;

(ii) a second RNA polymerase III promoter operably associated with a second RNA polymerase III termination signal; and

(iii) an insert disposed between the first and the second RNA polymerase III termination signals, wherein the first and second RNA polymerase III promoters are oriented to promote bidirectional transcription of the insert to produce siRNA molecules; and

(b) identifying at least one insert from which siRNA molecules are transcribed that affect a biological process of the one or more cells.

31. The method of Claim 30, wherein substantially all the expression vectors comprise a different insert.

32. The method of Claim 30, wherein all the expression vectors comprise a different insert.

33. A kit for creating an expression vector for producing siRNA molecules, comprising:

(a) an expression vector comprising:

(i) a first RNA polymerase III promoter operably associated with a first RNA polymerase III termination signal;

(ii) a second RNA polymerase III promoter operably associated with a second RNA polymerase III termination signal; and

(iii) a restriction enzyme cleavage site disposed within each of the first and second RNA polymerase III termination signals, wherein the first and second RNA polymerase III promoters are oriented to promote bidirectional transcription of an insert introduced between the restriction enzyme cleavage sites; and

(b) packaging.

34. A kit for creating an expression vector for producing siRNA molecules, comprising:

(a) a first primer for amplifying a sense strand of a nucleic acid molecule comprising a first RNA polymerase III promoter operably associated with a first RNA polymerase III termination signal;

(b) a second primer for amplifying an antisense strand of a nucleic acid molecule comprising a second RNA polymerase III promoter operably associated with a second RNA polymerase III termination signal;

(c) a double-stranded nucleic acid template comprising the first RNA polymerase III promoter or the second RNA polymerase III promoter; and

(d) packaging.